



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

May 13, 2003

MEMORANDUM:

Subject: Efficacy Review for EPA Reg. No.: 10492-4, "Isotex 70 Disinfecting Towelettes"
DP Barcode: D287746
Case No: 008014

From: Emily Mitchell, M.S., Team Leader *Emily Mitchell 5/15/03*
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Applicant: Palmero Health Care
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Formulation From Label:

<u>Active Ingredient(s)</u>	<u>% by wt.</u>
n-Alkyl (60% C ₁₄ , 30% C ₁₆ , 5% C ₁₂ , 5% C ₁₈)	
dimethyl benzyl ammonium chloride.....	0.12%
n-Alkyl (68% C ₁₂ , 32% C ₁₄)	
dimethyl ethyl benzyl ammonium chloride.....	0.12%
Isopropyl alcohol.....	63.25%
<u>Inert Ingredient(s)</u>	<u>36.51%</u>
Total	100.00%

I BACKGROUND

The product, Isotex 70 Disinfecting Towelettes (EPA Reg. No. 10492-4), is an EPA-approved disinfectant (bactericide, tuberculocide, virucide, fungicide) for use on hard, non-porous surfaces, including for use in hospitals, intensive care units, emergency medical settings, central supply, laboratories, clinics, nursing homes, and dental suites. The applicant requested an amendment to the registration of this product, as well as its product EPA Reg. No. 10492-5, to add claims of effectiveness against the Hepatitis B virus. All data to support the claim for Reg. No 10492-4 were circulated for review with the data package for the applicant's other amendment (assigned DP Barcode D287700; Action No. E153). Those studies were conducted at AppTec ATS located at 2540 Executive Drive in St. Paul, Minnesota 55120.

This data package contained correspondence from the applicant, EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-4 (Confidential Statement of Formula), the proposed label, and the last accepted label (dated November 19, 1998).

Note: EPA Form 8570-4 (Confidential Statement of Formula) contains Confidential Business Information. Data or information claimed by the applicant to be FIFRA confidential has not been included in this report.

II USE DIRECTIONS

The product is designed to be used for disinfecting pre-cleaned, hard, non-porous surfaces such as chairs, counter tops, drawer pulls, carts, baskets, tables, cabinets, telephones, and food-contact surfaces. Directions on the proposed label provided the following information regarding preparation and use of the product as a disinfectant: Completely pre-clean surfaces to be disinfected. The product can be used for this purpose. Thoroughly wet the surface with another towelette and allow to remain wet for 1 minute. When used as a tuberculocidal disinfectant, allow the surface to remain visibly wet for 5 full minutes at 20°C. Use on food-contact surfaces is required to be followed by a potable water rinse.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products Test (for spray disinfectants) must be used in developing data for virucides intended for use upon dry inanimate, environmental surfaces (e.g., floors, tables, cleaned dried medical instruments). To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of two different batches of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with

the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. These Agency standards are presented in DIS/TSS-7.

Virucides – Novel Virus Protocol Standards

To ensure that a virus protocol has been adequately validated, data should be provided from at least two independent laboratories for each product tested (i.e., two batches per product per laboratory). The validation of a protocol requires the use of a common positive control disinfectant to be tested concurrently with all new products. For the Hepatitis B Virus protocol, the usual control is BTC 835, a quaternary ammonium compound product obtained from Stepan Company. This agent serves as both an intra-laboratory and an inter-laboratory control and is used for analyzing the reproducibility of the efficacy data results for the protocol. These Agency standards are tailored from those presented in the Federal Register, Vol. 65, No. 166, Friday, August 25, 2000.

Disinfectants for Use on Hard Surfaces Using Pre-saturated or Impregnated Towelettes

Towelette products represent a unique combination of antimicrobial chemical and applicator, pre-packaged as a unit in fixed proportions. As such, the complete product, as offered for sale, should be tested according to the directions for use to ensure the product's effectiveness in disinfecting hard surfaces. The standard test methods available for hard surface disinfectants (i.e., AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method), if followed exactly, would not closely simulate the way a towelette product is used. Agency guidelines recommend that a simulated-use test be conducted by modifying the AOAC Germicidal Spray Products as Disinfectants Method. Agency guidelines further recommend that instead of spraying the inoculated surface of the glass slide, the product should be tested by wiping the surface of the glass slide with the saturated towelette, and then subculturing the slides after a specified holding time. Liquid expressed from the used towelette should also be subcultured. The above Agency standards are presented in DIS/TSS-1 and EPA Pesticide Assessment Guidelines, Subdivision G, §91-2(h), Pre-saturated or impregnated towelettes.

Confirmatory Efficacy Data Requirements

Under certain circumstances, an applicant is permitted to rely on previously submitted efficacy data to support an application or amendment for registration of a product and to submit only minimal confirmatory efficacy data on his own product to demonstrate his ability to produce an effective formulation. For a duplicated product formulation, the applicant manufactures a formulation that duplicates a product that is already registered with complete supporting efficacy data. The chemical composition, manufacturing procedure, label claims, and directions for use must be identical in substance to those of the original registration, and specific references to the supporting data developed for the original product must be furnished by the applicant. Confirmatory data must be developed on the applicant's own finished product. The above Agency standards are presented in DIS/TSS-5.

IV COMMENTS ON THE REFERENCED EFFICACY STUDIES

Note: No efficacy data were included in this package. Efficacy data for EPA Reg. No. 10492-5 were previously reviewed; the comments below are taken from the efficacy report prepared for the data package assigned DP Barcode D287700; Action No. E153.

1. MRID 458167-01 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Duck Hepatitis B Virus" for DisCide ULTRA Disinfecting Spray, by Karen M. Ramm. Study conducted at AppTec ATS. Study completion date – November 15, 2002.

This study, under the direction of Study Director Karen M. Ramm, was conducted against the Duck Hepatitis B virus (DHBV) (strain not specified; obtained from Hepadnavirus Testing Inc., Palo Alto, California) using primary duck hepatocytes (from ducklings obtained from the Abbendroth Hatchery, Waterloo, Wisconsin) as the host system. The study protocol followed guidelines set forth in the Federal Register: August 25, 2000 (Volume 65, Number 166). Two lots (Lot Nos. 14-04182A and 14-05152A) of the product, DisCide ULTRA Disinfecting Spray, were tested. The stock virus cultures contained 100% duck serum as the organic soil load. Two glass carriers were tested for each product lot against the target virus. Films of virus were made by spreading 0.2 mL of stock virus on the bottoms of separate sterilized glass Petri dishes. The virus films were dried for 30 minutes at 24°C. For each lot of product, the product was sprayed (4 pumps) onto the virus films at a distance of 12 inches, completely covering the virus films. The product was allowed to remain for 1 minute at 20°C. The virus-disinfectant mixture was scraped from the surface of the dish with a cell scraper. Each sample (2.0 mL) was loaded onto pre-spun Sephadex columns and spun to obtain the eluate. Columns of Sephadex LH-20-100 were equilibrated with phosphate buffered saline containing 1% albumin. Ten-fold serial dilutions were prepared, using Leibovitz-15 medium supplemented with 0.1% glucose, 10µM dexamethasone, 10µg/mL insulin, 20mM HEPES, 10µg/mL gentamicin, and 100 units/mL penicillin. To further aid in removing the cytotoxic effects of the product, the 10⁻² and 10⁻³ dilutions were passed through Sephadex columns a second time. Primary duck hepatocytes were inoculated in quadruplicate with 1.0 mL of each dilution. The cultures were incubated at 36-38°C in 5-7% CO₂ overnight for viral adsorption. The cells were then "re-fed" with 3.0 mL of the test medium, and returned to incubation conditions for 11 days. The cultures were observed microscopically for test substance cytotoxicity and the cells were fixed with ethanol. The plates were assayed by indirect immunofluorescence assay. Controls included virus stock titer, dried virus control, neutralizer effectiveness, cytotoxicity, and data consistency. BTC 835 (EPA Reg. No. 1839-32) was used as the data consistency control at two concentrations, 175 ppm and 350 ppm (titration results not provided). Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The log₁₀ reduction in infectivity was calculated using the revised EPA-approved method for calculating the Most Probable Number (MPN).

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Dried virus films were exposed to the common positive control product for 10 minutes at 20°C.

2. MRID 458167-02 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Duck Hepatitis B Confirmatory Assay" for DisCide ULTRA Disinfecting Spray, by Mary J. Miller. Study conducted at AppTec ATS. Study completion date – July 8, 2002.

This study, under the direction of Study Director Mary J. Miller, was conducted against the Duck Hepatitis B virus (strain not identified; Hepadnavirus Testing Inc., Palo Alto, California) using primary duck hepatocytes (from ducklings obtained from the Abendroth Hatchery, Waterloo, Wisconsin) as the host system. The study protocol followed guidelines set forth in the Federal Register: August 25, 2000 (Volume 65, Number 166). One lot (Lot No. 14-04182A) of the product, DisCide ULTRA Disinfecting Spray, was tested. The stock virus cultures contained 100% duck serum as the organic soil load. Two glass carriers were tested for the product lot against the target virus. Films of virus were made by spreading 0.2 mL of stock virus on the bottoms of separate sterilized glass Petri dishes. The virus films were dried for 30 minutes at 23°C. For the lot of product, the product was sprayed (4 pumps) onto the virus films at a distance of 12 inches, completely covering the virus films. The product was allowed to remain for 1 minute at 23°C. The virus-disinfectant mixture was scraped from the surface of the dish with a cell scraper. Each sample (2.0 mL) was loaded onto pre-spun Sephadex columns and spun to obtain the eluate. Columns of Sephadex LH-20-100 were equilibrated with phosphate buffered saline containing 1% albumin. Ten-fold serial dilutions were prepared, using Leibovitz-15 medium supplemented with 0.1% glucose, 10µM dexamethasone, 10µg/mL insulin, 20mM HEPES, 10µg/mL gentamicin, and 100 units/mL penicillin. Primary duck hepatocytes were inoculated in quadruplicate with 1.0 mL of each dilution. A 2.0 mL aliquot of test medium was added to each well. The cultures were incubated at 36-38°C in 5-7% CO₂ overnight for viral adsorption. The cells were then "re-fed" with 3.0 mL, and returned to incubation conditions for 10 days. The cultures were observed microscopically for test substance cytotoxicity and the cells were fixed with ethanol. The plates were assayed by indirect immunofluorescence assay. Controls included virus stock titer, dried virus control, neutralizer effectiveness, cytotoxicity, and data consistency. BTC 835 (EPA Reg. No. 1839-32) was used as the data consistency control at two concentrations, 175 ppm and 350 ppm (titration results not provided). Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The log₁₀ reduction in infectivity was calculated using the EPA-approved method for calculating the Most Probable Number (MPN).

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Dried virus films were exposed to the common positive control product for 10 minutes at 21°C.

Note: The applicant provided the data for a failed trial. In that trial, the dried virus control titer was below the required number (at least 10⁴). Thus, the test was invalid. These data were not used to evaluate efficacy of the test product. See Attachment I of the MRID study.

V RESULTS

Note: No efficacy data were included in this package. Efficacy data for EPA Reg. No. 10492-5 were previously reviewed; the results below are taken from the efficacy report prepared for the data package assigned DP Barcode D287700; Action No. E153.

MRID	Organism	Results - DisCide ULTRA Disinfecting Spray			Dried Virus Control (TCID ₅₀ /1.0 mL)
			Lot No. 14-04182A	Lot No. 14-05152A	
458167-01	Duck Hepatitis B virus	10 ⁻¹ to 10 ⁻⁴ dilutions	Complete inactivation	Complete inactivation	10 ^{4.75} , 10 ^{4.5}
		TCID ₅₀ /1.0 mL	≤ 10 ^{1.5}	≤ 10 ^{1.5}	
458167-02	Duck Hepatitis B virus	10 ⁻¹ to 10 ⁻⁴ dilutions	Complete inactivation		10 ^{4.5}
		TCID ₅₀ /1.0 mL	≤ 10 ^{1.5}		

MRID	Organism	Results - BTC 835			Dried Virus Control (TCID ₅₀ /1.0 mL)
			BTC 835, 175 ppm	BTC 835, 350 ppm	
458167-01	Duck Hepatitis B virus	10 ⁻¹ dilution	Complete inactivation	Complete inactivation	10 ^{4.75} , 10 ^{4.5}
		10 ⁻² dilution	Test virus present	Test virus present	
		10 ⁻³ dilution	Test virus present	Test virus present	
		10 ⁻⁴ dilution	Test virus present	Test virus present	
		TCID ₅₀ /1.0 mL	10 ^{4.25} , 10 ^{3.75}	10 ^{3.75} , 10 ^{4.0}	
458167-02	Duck Hepatitis B virus	10 ⁻¹ dilution	Complete inactivation	Complete inactivation	10 ^{4.5}
		10 ⁻² dilution	Test virus present	Test virus present	
		10 ⁻³ dilution	Test virus present	Complete inactivation	

MRID	Organism	Results - BTC 835			Dried Virus Control (TCID ₅₀ /1.0 mL)
			BTC 835, 175 ppm	BTC 835, 350 ppm	
		10 ⁻⁴ dilution	Complete inactivation	Complete inactivation	
		TCID ₅₀ /1.0 mL	10 ^{2.75} , 10 ^{2.5}	10 ^{1.75} , 10 ^{2.25}	

VI CONCLUSIONS

The previously reviewed efficacy data (MRID Nos. 458167-01 and 02; included with the data package assigned DP Barcode D287700; Action No. E153) do not currently support the use of the product, DisCide ULTRA Disinfecting Spray, as a disinfectant with virucidal activity when tested against Duck Hepatitis B virus (a surrogate for Human Hepatitis B virus) on hard, non-porous surfaces for a contact time of 1 minute. Although complete inactivation (no growth) was indicated in the 10⁻¹ through 10⁻⁴ dilutions of the product, DisCide ULTRA Disinfecting Spray, the common positive control product, BTC 835, did not behave as expected. The control product at 350 ppm failed to kill the test virus at all dilutions. The dried carrier count was at least 10⁴. The studies were performed at the same laboratory but under the direction of different study directors.

Note: The lot number of the control product, BTC 835, was not provided and the age of the control product is unknown. Dried virus films were exposed to the BTC 835 control product for 10 minutes; whereas, the dried virus films were exposed to the test product for 1 minute.

VII RECOMMENDATIONS

The proposed label claims (as supported by MRID Nos. 458167-01 and -02 included with DP Barcode D28770, Action No. E153) are not currently acceptable regarding the use of the product, Isotex 70 Disinfecting Towelettes, as a virucide against the Hepatitis B virus on hard, non-porous surfaces for a contact time of 1 minute. The registrant must submit the positive control data so the Agency can evaluate the data and assess the significance of the failure of the common positive control product at 350 ppm to kill the test virus at all dilutions. The applicant should also provide the manufacturing date/expiration date of the BTC 835 product lot used in the study:

The registrant must also provide the following information:

- information on the specific virus strain;
- a more detailed description of the challenge Duck Hepatitis B virus strain, concerning its virus stock preparation and titration; and
- a complete description of the infectivity assay using immunofluorescence.

Note to PM: If and when the efficacy data is acceptable, please inform the registrant that the ability to bridge data from a liquid to a towelette formulation may change in the future after there is an approved towelette protocol for HBV.